CLAIMS

1. An isolated nucleotide sequence encoding an ACC synthase enzyme of pineapple comprising the sequence of nucleotides as shown in FIG. 1.

2. An isolated nucleotide sequence encoding an ACC synthase enzyme of papaya comprising the sequence of nucleotides as shown in FIG. 2.

3. An isolated nucleotide sequence encoding an ACC synthase enzyme of papaya comprising the sequence of nucleotides as shown in FIG. 3.

4. An isolated nucleotide sequence encoding an ACC synthase enzyme of mango comprising the sequence of nucleotides as shown in FIG. 4.

5. An isolated nucleotide sequence encoding an ACC synthase enzyme of mango comprising the sequence of nucleotides as shown in FIG. 5.

6. An isolated nucleotide sequence as claimed in Claims 1-5 wherein said sequence is a homolog thereof.

7. An isolated nucleotide sequence as claimed in Claim 6 wherein said homolog is obtained from a fruit, fruit part or cell thereof.

8. An isolated nucleotide sequence as claimed in Claim 7 wherein said fruit, fruit part or cell thereof is obtained from a pineapple plant, a mango plant or a papaya plant.

9. A method of generating a transgenic variety of pineapple wherein fruit senescence is substantially inhibited, said method including the steps of introducing into a pineapple plant, or plant part or cell thereof a vector comprising the nucleotide sequence of FIG. 1 wherein said sequence is operably linked, in the sense orientation, to one or more regulatory nucleotide sequences, and growing said plant or plant part or cell thereof to generate the transgenic variety of pineapple.

10. A method of generating a transgenic variety of pineapple wherein

fruit senescence is substantially inhibited, said method including the steps of introducing into a pineapple plant, or plant part or cell thereof a vector comprising the nucleotide sequence of FIG. 1 wherein said sequence is operably linked, in the antisense orientation, to one or more regulatory nucleotide sequences, and growing said plant or plant part or cell thereof to generate the transgenic variety of pineapple.

11. A method as claimed in Claim 9 ~~or Claim 10~~ wherein said nucleotide sequence is a homolog thereof.

12. A method of generating a transgenic variety of papaya wherein fruit senescence is substantially inhibited, said method including the steps of introducing into a papaya plant, or plant part or cell thereof a vector comprising the nucleotide sequence of FIG. 2 and/or FIG. 3 wherein said sequence is operably linked, in the sense orientation, to one or more regulatory nucleotide sequences, and growing said plant or plant part or cell thereof to generate the transgenic variety of papaya.

13. A method of generating a transgenic variety of papaya wherein fruit senescence is substantially inhibited, said method including the steps of introducing into a papaya plant, or plant part or cell thereof a vector comprising the nucleotide sequence of FIG. 2 and/or FIG. 3 wherein said sequence is operably linked, in the antisense orientation, to one or more regulatory nucleotide sequences, and growing said plant or plant part or cell thereof to generate the transgenic variety of papaya.

14. A method as claimed in Claim 12 ~~or Claim 13~~ wherein said nucleotide sequence is a homolog thereof.

15. A method of generating a transgenic variety of mango wherein fruit senescence is substantially inhibited, said method including the steps of introducing into a mango plant, or plant part or cell thereof a vector comprising the nucleotide sequence of FIG. 4 and/or FIG. 5 wherein said sequence is operably linked, in the sense orientation, to one or more regulatory nucleotide sequences, and growing said plant or plant part or cell thereof to generate the transgenic variety of mango.

16. A method of generating a transgenic variety of mango wherein fruit senescence is substantially inhibited, said method including the steps of introducing into a mango plant, or plant part or cell thereof a vector comprising the nucleotide sequence of FIG. 4 and/or FIG. 5 wherein said sequence is operably linked, in the antisense orientation, to one or more regulatory nucleotide sequences, and growing said plant or plant part or cell thereof to generate the transgenic variety of mango.

17. A method as claimed in Claim 15 ~~or Claim 16~~ wherein said nucleotide sequence is a homolog thereof.

18. ~~A vector which~~ contains at least one copy of a nucleotide sequence as claimed in any of Claims ~~1-8~~.

19. A vector as claimed in Claim 18 wherein said nucleotide sequence is under the control of one or more regulatory sequences.

20. A transgenic variety of pineapple when produced by the method of Claim 9 ~~or Claim 10~~.

21. A transgenic variety of papaya when produced by the method of Claim 12 ~~or Claim 13~~.

22. A transgenic variety of mango when produced by the method of Claim 15 or Claim 16.

23. A method of isolating at least a fragment of an ACC synthase gene involved in regulation of fruit ripening, said method including the steps of:-

(a) lysing ripened tissue of a fruit to produce a lysate;

(b) separating protein and carbohydrates from the lysate to produce an extract comprising substantially intact RNA wherein said extract is substantially incapable of inhibiting cDNA synthesis;

(c) reverse transcribing the RNA of said extract with an antisense primer complementary to a portion of said ACC synthase gene to synthesize a cDNA; and

(d) subjecting the cDNA to PCR with a sense primer and an antisense primer which are complementary to different portions of said

ACC synthase gene to amplify said at least a fragment of said ACC synthase gene.

24. A method as claimed in Claim 23 wherein the fruit is pineapple, papaya or mango fruit.

25. A method as claimed in Claim 23 wherein the step of lysing is effected by lysing the ripened tissue in a medium comprising 150 mM Tris pH 7.5 with boric acid, 2% SDS, 50 mM EDTA, 1% mercaptoethanol.

26. A method as claimed in Claim 23 wherein the step of separating is effected by at least one chloroform-isoamyl alcohol extraction of the lysate followed by at least two phenol-chloroform extractions of an aqueous phase resulting from the at least one chloroform-isoamyl alcohol extraction.

27. A method as claimed in Claim 23 wherein the step of separating is effected by at least one chloroform-isoamyl alcohol extraction of the lysate followed by at least two phenol-chloroform-isoamyl alcohol extractions of an aqueous phase resulting from the at least one chloroform-isoamyl alcohol extraction.

28. A method as claimed in Claim 23 wherein the sense primer used for step (c) as well as the sense primer and the antisense primer used for step (d) are degenerate primers.

29. A method as claimed in Claim 28 wherein said degenerate primers correspond to conserved portions of different ACC synthase isoforms.

30. A method as claimed in Claim 28 wherein said degenerate primers are selected from the group consisting of:

5' TA(C/T)TT(C/T)GA(C/T)GG(A/C/G/T)TGGAA(A/G)GC 3'; 11

5' TC(A/G)TCCAT(A/G)TT(A/C/G/T)GC(A/G)AA(A/G)CA 3'; 12

5' CA(A/G)ATGGG(A/C/G/T)(C/T)T(A/C/G/T)GC(A/C/G/T)GA(A/G)AA 3'; 13

5' AC(A/C/G/T)C(G/T)(A/G)AACCA(A/C/G/T)CC(A/C/G/T)GG(C/T)TC 3'; 15

5' GCTCTAGATA(C/T)TT(C/T)GA(C/T)GG(A/C/G/T)TGGAA(A/G)GC 3'; 16

5' GCGAATTC(A/G)TCCAT(A/G)TT(A/C/G/T)GC(A/G)AA(A/G)CA 3'; 17

5' CCTGATCA(A/G)ATGGG(A/C/G/T)(C/T)T(A/C/G/T)GC(A/C/G/T)GA(A/G)AA 3'; and

5' CTCTGCAGC(A/G)AA(A/G)CA(A/C/G/T)AC(A/C/G/T)C(G/T)(A/G)AA CCA 3'.

31. A method as claimed in Claim 23 wherein the fruit is pineapple, the antisense primer in step (c) is 5' TC(A/G)TCCAT(A/G)TT (A/C/G/T)GC(A/G)AA(A/G)CA 3', the sense primer for step (e) is 5' TA(C/T)TT(C/T)GA(C/T)GG(A/C/G/T)TGGAA(A/G)GC 3' or 5' CA(A/G)ATGGG(A/C/G/T)(C/T)T(A/C/G/T)GC(A/C/G/T)GA(A/G)AA 3', and the antisense primer for step (d) is 5' TC(A/G)TCCAT(A/G)TT(A/C/G/T)GC(A/G)AA(A/G)CA 3' or 5' AC(A/C/G/T)C(G/T)(A/G)AACCA(A/C/G/T)CC(A/C/G/T)GG(C/T)TC 3'.

32. A method as claimed in Claim 23 wherein the fruit is mango or papaya, the antisense primer for step (c) is 5' GCGAATTC(A/G)TCCAT(A/G)TT(A/C/G/T)GC(A/G)AA(A/G)CA 3', the sense primer for step (e) is 5' GCTCTAGATA(C/T)TT(C/T)GA(C/T)GG (A/C/G/T)TGGAA(A/G)GC 3' or 5' CCTGATCA(A/G)ATGGG (A/C/G/T)(C/T)T(A/C/G/T)GC(A/C/G/T)GA(A/G)AA 3', and the antisense primer for step (d) is 5' GCGAATTC(A/G)TCCAT(A/G)TT(A/C/G/T)GC (A/G)AA(A/G)CA 3' or 5' CTCTGCAGC(A/G)AA(A/G)CA(A/C/G/T)AC (A/C/G/T)C(G/T)(A/G)AACCA 3'.

33. A method as claimed in Claim 23 wherein step (d) comprises a first PCR and a second PCR wherein ~~the second PCR employs a nested pair~~ of primers relative to those utilized in the first PCR.

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